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CONTENTS/SUMMARIES

Regulation of Gene Expression by Oxygen in *Saccharomyces cerevisiae*. Richard S. Zitomer and Charles V. Lowry 1-11

Summary: The oxygen regulation of two broad categories of yeast genes is discussed in this review. The first is made up of genes regulated by heme, and the second is made up of genes whose regulation is heme independent. Heme-regulated genes fall into two classes: heme-activated and heme-repressed genes. Activation is achieved through one of two transcriptional activators, the heme-dependent HAP1 protein or the heme-activated, glucose-repressed HAP2/3/4 complex. Some of the properties and the DNA-binding sites of these activators are discussed. Heme repression is achieved through the action of the ROX1 repressor, the expression of which is transcriptionally activated by heme. Once ROX1 is synthesized, its function is heme independent. Evidence that ROX1 binds to DNA or is part of a DNA-binding complex is described. Factors which modulate the function of these regulatory proteins are discussed, and a schematic of heme activation and repression is presented. The mitochondrial subunits of cytochrome c oxidase are induced by oxygen in a heme-independent fashion. The translation of one, cytochrome c oxidase subunit III, is dependent upon three nucleus-encoded initiation factors. One of these, PET494, is itself translationally regulated by oxygen in a heme-independent fashion. The expression of at least four other mitochondrially encoded cytochrome subunits is dependent upon specific translation factors, raising the potential for translational regulation as a general mechanism. Finally, a number of anaerobic genes that show heme-independent, oxygen-repressed expression have been identified. These fall into two kinetic classes, suggesting that there are at least two different regulatory circuitries.

Two-Way Chemical Signaling in *Agrobacterium*-Plant Interactions. Stephen C. Winans 12-31

Summary: The discovery in 1977 that *Agrobacterium* species can transfer a discrete segment of oncogenic DNA (T-DNA) to the genome of host plant cells has stimulated an intense interest in the molecular biology underlying these plant-microbe associations. This attention in turn has resulted in a series of insights about the biology of these organisms that continue to accumulate at an ever-increasing rate. This excitement was due in part to the notion that this unprecedented interkingdom DNA transfer could be exploited to create transgenic plants containing foreign genes of scientific or commercial importance. In the course of these discoveries, *Agrobacterium* became one of the best available models for studying the molecular interactions between bacteria and higher organisms. One extensively studied aspect of this association concerns the exchange of chemical signals between *Agrobacterium* spp. and host plants. *Agrobacterium* spp. can recognize no fewer than five classes of low-molecular-weight com-

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pounds released from plants, and other classes probably await discovery. The most widely studied of these are phenolic compounds, which stimulate the transcription of the genes needed for infection. Other compounds include specific monosaccharides and acidic environments which potentiate vir gene induction, acidic polysaccharides which induce one or more chromosomal genes, and a family of compounds called opines which are released from tumorous plant cells to the bacteria as nutrient sources. *Agrobacterium* spp. in return release a variety of chemical compounds to plants. The best understood is the transferred DNA itself, which contains genes that in various ways upset the balance of phytohormones, ultimately causing neoplastic cell proliferation. In addition to transferring DNA, some *Agrobacterium* strains directly secrete phytohormones. Finally, at least some strains release a pectinase, which degrades a component of plant cell walls.

Virulence Factors of the Family *Legionellaceae*. John N. Dowling,
Asish K. Saha, and Robert H. Glew 32-60

Summary: Whereas bacteria in the genus Legionella have emerged as relatively frequent causes of pneumonia, the mechanisms underlying their pathogenicity are obscure. The legionellae are facultative intracellular pathogens which multiply within the phagosome of mononuclear phagocytes and are not killed efficiently by polymorphonuclear leukocytes. The functional defects that might permit the intracellular survival of the legionellae have remained an enigma until recently. Phagosome-lysosome fusion is inhibited by a single strain (Philadelphia 1) of Legionella pneumophila serogroup 1, but not by other strains of L. pneumophila or other species. It has been found that following the ingestion of Legionella organisms, the subsequent activation of neutrophils and monocytes in response to both soluble and particulate stimuli is profoundly impaired and the bactericidal activity of these cells is attenuated, suggesting that Legionella bacterial cell-associated factors have an inhibitory effect on phagocyte activation. Two factors elaborated by the legionellae which inhibit phagocyte activation have been described. First, the Legionella (cyto)toxin blocks neutrophil oxidative metabolism in response to various agonists by an unknown mechanism. Second, L. micdadei bacterial cells contain a phosphatase which blocks superoxide anion production by stimulated neutrophils. The Legionella phosphatase disrupts the formation of critical intracellular second messengers in neutrophils. In addition to the toxin and phosphatase, several other moieties that may serve as virulence factors by promoting cell invasion or intracellular survival and multiplication are elaborated by the legionellae. Molecular biological studies show that a cell surface protein named Mip is necessary for the efficient invasion of monocytes. A possible role for a Legionella phospholipase C as a virulence factor is still largely theoretical. L. micdadei contains an unusual protein kinase which catalyzes the phosphorylation of eukaryotic substrates, including phosphatidylinositol and tubulin. Since the phosphorylation of either phosphatidylinositol or tubulin might compromise phagocyte activation and bactericidal functions, this enzyme may well be a virulence factor. Administration of the L. pneumophila exoprotease induces lesions resembling those of Legionella pneumonia and kills guinea pigs, suggesting that this protein plays a role in the pathogenesis of legionellosis. However, recent work with a genetically engineered strain has convincingly shown that the protease is not necessary for intracellular survival or virulence. As might be expected with a complex process like intracellular parasitism, it appears that the capability of Legionella strains to invade and multiply in host phagocytes is multifactorial and that no single moiety which is responsible for the virulence phenotype will be found.

RNA Recombination in Animal and Plant Viruses. Michael M. C.
Lai 61-79

Summary: An increasing number of animal and plant viruses have been shown to undergo RNA-RNA recombination, which is defined as the exchange of genetic information between nonsegmented RNAs. Only some of these viruses have been shown to undergo recombination in experimental infection of tissue culture, animals, and plants. However, a survey of viral RNA structure and sequences suggests that many RNA viruses were derived from homologous or nonhomologous recombination between

viruses or between viruses and cellular genes during natural viral evolution. The high frequency and widespread nature of RNA recombination indicate that this phenomenon plays a more significant role in the biology of RNA viruses than was previously recognized. Three types of RNA recombination are defined: homologous recombination; aberrant homologous recombination, which results in sequence duplication, insertion, or deletion during recombination; and nonhomologous (illegitimate) recombination, which does not involve sequence homology. RNA recombination has been shown to occur by a copy choice mechanism in some viruses. A model for this recombination mechanism is presented.

Properties and Use of Botulinum Toxin and Other Microbial

Neurotoxins in Medicine. Edward J. Schantz and Eric A. Johnson

80-99

Summary: Crystalline botulinum toxin type A was licensed in December 1989 by the Food and Drug Administration for treatment of certain spasmodic muscle disorders following 10 or more years of experimental treatment on human volunteers. Botulinum toxin exerts its action on a muscle indirectly by blocking the release of the neurotransmitter acetylcholine at the nerve ending, resulting in reduced muscle activity or paralysis. The injection of only nanogram quantities (1 ng = 30 mouse 50% lethal doses [U]) of the toxin into a spastic muscle is required to bring about the desired muscle control. The type A toxin produced in anaerobic culture and purified in crystalline form has a specific toxicity in mice of 3×10^7 U/mg. The crystalline toxin is a high-molecular-weight protein of 900,000 M_r and is composed of two molecules of neurotoxin (ca. 150,000 M_r) noncovalently bound to nontoxic proteins that play an important role in the stability of the toxic unit and its effective toxicity. Because the toxin is administered by injection directly into neuromuscular tissue, the methods of culturing and purification are vital. Its chemical, physical, and biological properties as applied to its use in medicine are described. Dilution and drying of the toxin for dispensing causes some detoxification, and the mouse assay is the only means of evaluation for human treatment. Other microbial neurotoxins may have uses in medicine; these include serotypes of botulinum toxins and tetanus toxin. Certain neurotoxins produced by dinoflagellates, including saxitoxin and tetrodotoxin, cause muscle paralysis through their effect on the action potential at the voltage-gated sodium channel. Saxitoxin used with anaesthetics lengthens the effect of the anaesthetic and may enhance the effectiveness of other medical drugs. Combining toxins with drugs could increase their effectiveness in treatment of human disease.

Cyclic AMP in Prokaryotes. James L. Botsford and James G. Harman

100-122

Summary: Cyclic AMP (cAMP) is found in a variety of prokaryotes including both eubacteria and archaebacteria. cAMP plays a role in regulating gene expression, not only for the classic inducible catabolic operons, but also for other categories. In the enteric coliforms, the effects of cAMP on gene expression are mediated through its interaction with and allosteric modification of a cAMP-binding protein (CRP). The CRP-cAMP complex subsequently binds specific DNA sequences and either activates or inhibits transcription depending upon the positioning of the complex relative to the promoter. Enteric coliforms have provided a model to explore the mechanisms involved in controlling adenylate cyclase activity, in regulating adenylate cyclase synthesis, and in performing detailed examinations of CRP-cAMP complex-regulated gene expression. This review summarizes recent work focused on elucidating the molecular mechanisms of CRP-cAMP complex-mediated processes. For other bacteria, less detail is known. cAMP has been implicated in regulating antibiotic production, phototrophic growth, and pathogenesis. A role for cAMP has been suggested in nitrogen fixation. Often the only data that support cAMP involvement in these processes includes cAMP measurement, detection of the enzymes involved in cAMP metabolism, or observed effects of high concentrations of the nucleotide on cell growth.

DNA Looping. Kathleen S. Matthews 123-136

Summary: DNA-looping mechanisms are part of networks that regulate all aspects of DNA metabolism, including transcription, replication, and recombination. DNA looping is involved in regulation of transcriptional initiation in prokaryotic operons, including ara, gal, lac, and deo, and in phage systems. Similarly, in eukaryotic organisms, the effects of enhancers appear to be mediated at least in part by loop formation, and examples of DNA looping by hormone receptor proteins and developmental regulatory proteins have been found. In addition, instances of looped structures have been found in replication and in recombination in both prokaryotes and eukaryotes. DNA loop formation may have different functions in different cellular contexts; in some cases, the loop itself is requisite for regulation, while in others the increase in the effective local concentration of protein may account for the effects observed. The ability of DNA to form loops is affected by the distance between binding sites; by the DNA sequence, which determines deformability and bendability; and by the presence of other proteins that exert an influence on the conformation of a particular sequence. Alteration of the stability of DNA loops and/or protein-DNA binding by extra- or intracellular signals provides responsivity to changing metabolic or environmental conditions. The fundamental property of site-specific protein binding to DNA can be combined with protein-protein and protein-ligand interaction to generate a broad range of physiological states.

Correlation of a Subset of the pLC Plasmids to the Physical Map of *Escherichia coli* K-12. Akiko Nishimura, Kiyotaka Akiyama, Yuji Kohara, and Kensuke Horiuchi 137-151

Summary: We determined map positions of the Escherichia coli K-12 portions of a subset of the hybrid E. coli-ColE1 plasmids constructed by Clarke and Carbon. The probe DNA of pLC plasmids was labeled with digoxigenine-dUTP, hybridized to the 476 phage clones of the E. coli ordered clone bank miniset, which was adsorbed on a strip of nylon membrane filters, and detected by enzyme-linked immunoassay and a subsequent enzyme-catalyzed color reaction. The total number of Clarke-Carbon plasmids we analyzed was 518, for which chromosomal locations of 297 clones were newly determined in the present study. Another 180 plasmids gave results that agreed with those reported previously, and the remaining 41 plasmids gave map positions different from those described in the previous report. A chromosome map of E. coli which shows the locations of 518 pLC plasmids on it is presented, as well as a table which correlates the pLC plasmids with the clones of the E. coli ordered clone bank miniset on the basis of the hybridization data. We estimate that approximately one-half of the entire genome of E. coli was covered by the pLC plasmids used in this study.

Evolution and Ecology of Influenza A Viruses. Robert G. Webster, William J. Bean, Owen T. Gorman, Thomas M. Chambers, and Yoshihiro Kawaoka..... 152-179

Summary: In this review we examine the hypothesis that aquatic birds are the primordial source of all influenza viruses in other species and study the ecological features that permit the perpetuation of influenza viruses in aquatic avian species. Phylogenetic analysis of the nucleotide sequence of influenza A virus RNA segments coding for the spike proteins (HA, NA, and M2) and the internal proteins (PB2, PB1, PA, NP, M, and NS) from a wide range of hosts, geographical regions, and influenza A virus subtypes support the following conclusions. (i) Two partly overlapping reservoirs of influenza A viruses exist in migrating waterfowl and shorebirds throughout the world. These species harbor influenza viruses of all the known HA and NA subtypes. (ii) Influenza viruses have evolved into a number of host-specific lineages that are exemplified by the NP gene and include equine Prague/56, recent equine strains, classical swine and human strains, H13 gull strains, and all other avian strains. Other genes show similar patterns, but with extensive evidence of genetic reassortment. Geographical as well as host-specific lineages are evident. (iii) All of the influenza A viruses of mammalian sources originated from the avian gene pool, and it is possible

that influenza B viruses also arose from the same source. (iv) The different virus lineages are predominantly host specific, but there are periodic exchanges of influenza virus genes or whole viruses between species, giving rise to pandemics of disease in humans, lower animals, and birds. (v) The influenza viruses currently circulating in humans and pigs in North America originated by transmission of all genes from the avian reservoir prior to the 1918 Spanish influenza pandemic; some of the genes have subsequently been replaced by others from the influenza gene pool in birds. (vi) The influenza virus gene pool in aquatic birds of the world is probably perpetuated by low-level transmission within that species throughout the year. (vii) There is evidence that most new human pandemic strains and variants have originated in southern China. (viii) There is speculation that pigs may serve as the intermediate host in genetic exchange between influenza viruses in avian and humans, but experimental evidence is lacking. (ix) Once the ecological properties of influenza viruses are understood, it may be possible to interdict the introduction of new influenza viruses into humans.

Sexual Agglutination in Budding Yeasts: Structure, Function, and Regulation of Adhesion Glycoproteins. Peter N. Lipke and Janet Kurjan

180-194

Summary: The sexual agglutinins of the budding yeasts are cell adhesion proteins that promote aggregation of cells during mating. In each yeast species, complementary agglutinins are expressed by cells of opposite mating type that interact to mediate aggregation. *Saccharomyces cerevisiae* α -agglutinin and its analogs from other yeasts are single-subunit glycoproteins that contain N-linked and O-linked oligosaccharides. The N-glycosidase-sensitive carbohydrate is not necessary for activity. The proposed binding domain of α -agglutinin has features characteristic of the immunoglobulin fold structures of cell adhesion proteins of higher eukaryotes. The C-terminal region of α -agglutinin plays a role in anchoring the glycoprotein to the cell surface. The *S. cerevisiae* α -agglutinin and its analogs from other species contain multiple subunits; one or more binding subunits, which interact with the opposite agglutinin, are disulfide bonded to a core subunit, which mediates cell wall anchorage. The core subunits are composed of 80 to 95% O-linked carbohydrate. The binding subunits have less carbohydrate, and both carbohydrate and peptide play roles in binding. The α -agglutinin and α -agglutinin genes from *S. cerevisiae* have been cloned and shown to be regulated by the mating-type locus, MAT, and by pheromone induction. The agglutinins are necessary for mating under conditions that do not promote cell-cell contact. The role of the agglutinins therefore is to promote close interactions between cells of opposite mating type and possibly to facilitate the response to pheromone, thus increasing the efficiency of mating. We speculate that they mediate enhanced response to sex pheromones by providing a synapse at the point of cell-cell contact, at which both pheromone secretion and cell fusion occur.

Gene Regulation of Plasmid- and Chromosome-Determined Inorganic Ion Transport in Bacteria. Simon Silver and Mark Walderhaug

195-228

Summary: Regulation of chromosomally determined nutrient cation and anion uptake systems shows important similarities to regulation of plasmid-determined toxic ion resistance systems that mediate the outward transport of deleterious ions. Chromosomally determined transport systems result in accumulation of K^+ , Mg^{2+} , Fe^{3+} , Mn^{2+} , PO_4^{3-} , SO_4^{2-} , and additional trace nutrients, while bacterial plasmids harbor highly specific resistance systems for AsO_2^- , AsO_4^{3-} , CrO_4^{2-} , Cd^{2+} , Co^{2+} , Cu^{2+} , Hg^{2+} , Ni^{2+} , SbO_2^- , TeO_3^{2-} , Zn^{2+} , and other toxic ions. To study the regulation of these systems, we need to define both the trans-acting regulatory proteins and the cis-acting target operator DNA regions for the proteins. The regulation of gene expression for K^+ and PO_4^{3-} transport systems involves two-component sensor-effector pairs of proteins. The first protein responds to an extracellular ionic (or related) signal and then transmits the signal to an intracellular DNA-binding protein. Regulation of Fe^{3+} transport utilizes the single iron-binding and DNA-binding protein Fur. The MerR regulatory protein for mercury resistance both represses and activates transcription. The ArsR regulatory protein functions as a repressor for the arsenic and antimony(III) efflux system.

Although the predicted *cadR* regulatory gene has not been identified, cadmium, lead, bismuth, zinc, and cobalt induce this system in a carefully regulated manner from a single mRNA start site. The *cadA* Cd^{2+} resistance determinant encodes an $\text{E}_1\text{I-E}_2$ -class efflux ATPase (consisting of two polypeptides, rather than the one earlier identified). Cadmium resistance is also conferred by the *czc* system (which confers resistances to zinc and cobalt in *Alcaligenes* species) via a complex efflux pump consisting of four polypeptides. These two cadmium efflux systems are not otherwise related. For chromate resistance, reduced cellular accumulation is again the resistance mechanism, but the regulatory components are not identified. For other toxic heavy metals (with few exceptions), there exist specific plasmid resistances that remain relatively terra incognita for future exploration of bioinorganic molecular genetics and gene regulation.

Recent Evidence for Evolution of the Genetic Code. Syozo Osawa,
Thomas H. Jukes, Kimitsuna Watanabe, and Akira Muto

229–264

Summary: The genetic code, formerly thought to be frozen, is now known to be in a state of evolution. This was first shown in 1979 by Barrell et al. (G. Barrell, A. T. Bankier, and J. Drouin, Nature [London] 282:189–194, 1979), who found that the universal codons AUA (isoleucine) and UGA (stop) coded for methionine and tryptophan, respectively, in human mitochondria. Subsequent studies have shown that UGA codes for tryptophan in Mycoplasma spp. and in all nonplant mitochondria that have been examined. Universal stop codons UAA and UAG code for glutamine in ciliated protozoa (except Euplotes octacarinatus) and in a green alga, Acetabularia. E. octacarinatus uses UAA for stop and UGA for cysteine. Candida species, which are yeasts, use CUG (leucine) for serine. Other departures from the universal code, all in nonplant mitochondria, are CUN (leucine) for threonine (in yeasts), AAA (lysine) for asparagine (in platyhelminths and echinoderms), UAA (stop) for tyrosine (in planaria), and AGR (arginine) for serine (in several animal orders) and for stop (in vertebrates). We propose that the changes are typically preceded by loss of a codon from all coding sequences in an organism or organelle, often as a result of directional mutation pressure, accompanied by loss of the tRNA that translates the codon. The codon reappears later by conversion of another codon and emergence of a tRNA that translates the reappeared codon with a different assignment. Changes in release factors also contribute to these revised assignments. We also discuss the use of UGA (stop) as a selenocysteine codon and the early history of the code.